

Figure 2B depicts results of transcription assays using rapalog 96, synthesized as described herein, as the dimerizer. The rapalog was tested in cells expressing a mutant FRB in which Thr 2098 was replaced by Leu. A rapamycin control is shown.

Figure 2C depicts results of transcription assays using rapalogs 42, 53 and 69, synthesized as described herein, as the dimerizers. The rapalogs were tested in cells expressing wild-type FRB. A rapamycin control is shown.

Figure 2D depicts results of transcription assays using rapalogs 42, 53 and 69, synthesized as described herein, as the dimerizers. The rapalogs were tested in cells expressing a mutant FRB in which Thr 2098 was replaced by Leu. A rapamycin control is shown.

Figure 2E depicts results of transcription assays using rapalogs 42, 53 and 69, synthesized as described herein, as the dimerizers. The rapalogs were tested in cells expressing a mutant FRB in which Thr 2098 was replaced by Phe. A rapamycin control is shown.

***On page 58, line 28, extending to page 59, line 18, replace the paragraph in the specification with the following;***

#### **Other components, design features and applications**

The chimeric proteins may contain as a heterologous domain a cellular localization domain such as a membrane retention domain. See e.g. PCT/US94/01617, especially pages 26-27. Briefly, a membrane retention domain can be isolated from any convenient membrane-bound protein, whether endogenous to the host cell or not. The membrane retention domain may be a transmembrane retention domain, i.e., an amino acid sequence which extends across the membrane as in the case of cell surface proteins, including many receptors. The transmembrane peptide sequence may be extended to span part or all of an extracellular and/or intracellular domain as well. Alternatively, the membrane retention domain may be a lipid membrane retention domain such as a myristoylation or palmitoylation site which permits association with the lipids of the cell surface membrane. Lipid membrane retention domains will usually be added at the 5' end of the coding sequence for N-terminal

binding to the membrane and, proximal to the 3' end for C-terminal binding. Peptide sequences involving post-translational processing to provide for lipid membrane binding are described by Carr, et al., PNAS USA (1988) 79, 6128; Aitken, et al., FEBS Lett. (1982) 150, 314; Henderson, et al., PNAS USA (1983) 80, 319; Schulz, et al., Virology (1984), 123, 2131; Dellman, et al., Nature (1985) 314, 374; and reviewed in Ann. Rev. of Biochem. (1988) 57, 69. An amino acid sequence of interest includes the sequence M-G-S-S-K-S-K-P-K-D-P-S-Q-R (SEQ ID NO 1). Various DNA sequences can be used to encode such sequences in the various chimeric proteins of this invention. Other localization domains include organelle-targeting domains and sequences such as -K-D-E-L (SEQ ID NO 2) and -H-D-E-L (SEQ ID NO 3) which target proteins bearing them to the endoplasmic reticulum, as well as nuclear localization sequences which are particularly useful for chimeric proteins designed for (direct) transcriptional regulation. Various cellular localization sequences and signals are well known in the art.

***On page 112, lines 5 - 20, replace the text in the specification with the following;***

*(f) Primer sequences*

1	5' GCATGTCTAGAGAGATGTGGCATGAAGGCCTGGAAG	( <u>SEQ ID NO 4</u> )
2	5' GCATCACTAGTCTTTGAGATTCGTCGGAACACATG	( <u>SEQ ID NO 5</u> )
3	5' GCACATTCTAGAATTGATACGCCAGACCCTTG	( <u>SEQ ID NO 6</u> )
4	5' CGATCAACTAGTAAGTGTCAATTTCCGGGGCCT	( <u>SEQ ID NO 7</u> )
5	5' GCACTATCTAGACTGAAGAACATGTGTGAGCACAGC	( <u>SEQ ID NO 8</u> )
6	5' GCACTATCTAGAGTGAGCGAGGAGCTGATCCGAGTG	( <u>SEQ ID NO 9</u> )
7	5' CGATCAACTAGTGGAACATATTGCAGCTCTAAGGA	( <u>SEQ ID NO 10</u> )
8	5' CGATCAACTAGTTGGCACAGCCAATTCAAGGTCCCG	( <u>SEQ ID NO 11</u> )
9	5' ATGCTCTAGACTGGGGGCCTTGCTTGGCAAC	( <u>SEQ ID NO 12</u> )
10	5' ATGCTCTAGAGATGAGTTTCCCACCATGGTG	( <u>SEQ ID NO 13</u> )
11	5' GCATGGATCCGCTCAACTAGTGGAGCTGATCTGACTCAG	( <u>SEQ ID NO 14</u> )
12	5' ATGCTCTAGACTTGAACCGGACCTGCCGCC	( <u>SEQ ID NO 15</u> )
13	5' GCATCACTAGTCCAGAAAGGGCACCAGCCAATAT	( <u>SEQ ID NO 16</u> )

Restriction sites are [underlined ( )Xbal = TCTAGA, SpeI = ACGAGT, BamHI = GGATCC( )].

***On page 113, lines 15 - 34, replace the contiguous sections in the specification with the following contiguous sections;***

Primer 1 5'-GCCATGGTGGCTAGCCTATAGTGAG (SEQ ID NO 17)

Primer2 5'-GGCGGTGTTGGCTAGCGTCGGTCAG (SEQ ID NO 18)

pSMTN2 contains unique EcoRI and HindIII sites downstream of the LTR. To facilitate cloning of transcription factor fusion proteins synthesized as XbaI-BamHI fragments the following sequence was inserted between the EcoRI and HindIII sites to create pSMTN3:

12CA5 epitope

5' gaattccagaagcgcgt ATG GCT TCT AGC TAT CCT TAT GAC GTG CCT GAC  
EcoRI

SV40 T NLS  
Y A S L G G P S S P K K K R K  
TAT GCC AGC CTG GGA GGA CCT TCT AGT CCT AAG AAG AAG AGA AAG

V (SEQ ID NO 19)

GTG TCT AGA TAT CGA GGA TCC CAA GCT T (SEQ ID NO 20)  
XbaI BamHI HindIII

***On page 130, lines 12 - 15, replace the text in the specification with the following;***

His6 HA tag\_\_\_\_\_FKBP->  
MHHHHHHYPYDVPDYAAMAHMGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSR  
DRNKPFFKFMLGKQEVIWGVEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIIPPHATLVFDV  
ELLKLE (SEQ ID NO 21)

***On page 131, lines 11 - 33, replace the 2 contiguous sections in the specification with the following 2 contiguous sections;***

Mutants designed for binding to C24 rapalogs:

Phe46His	agcataaaacttaTGgggcttgtttctg	(1)	<a href="#">(SEQ ID NO 22)</a>
Phe46Leu	agcataaaacttTaagggcttgtttctg	(2)	<a href="#">(SEQ ID NO 23)</a>
Phe46Ala	agcataaaacttaGCgggcttgtttctg	(3)	<a href="#">(SEQ ID NO 24)</a>
Phe48His	ttgcctagcataTGcttaaagggttg	(4)	<a href="#">(SEQ ID NO 25)</a>
Phe48Leu	ttgcctagcatTaacttaaagggttg	(5)	<a href="#">(SEQ ID NO 26)</a>
Phe48Ala	ttgcctagcataGCcttaaagggttg	(6)	<a href="#">(SEQ ID NO 27)</a>
Glu54Ala	cctcggatcaccGCctgcttgctag	(7)	<a href="#">(SEQ ID NO 28)</a>
Val55Ala	cagcctcggatcGCctctgcttgcc	(8)	<a href="#">(SEQ ID NO 29)</a>

Mutants designed for binding to C13/C14 rapalogs:

Phe36Ala	ccgggaggaatcGCtttcttccatcttc	(9)	<a href="#">(SEQ ID NO 30)</a>
Phe36Val	ccgggaggaatcGACtttcttccatcttc	(10)	<a href="#">(SEQ ID NO 31)</a>
Phe36Ser	ccgggaggaatcAGAttcttccatcttc	(11)	<a href="#">(SEQ ID NO 32)</a>
Phe36Met	ccgggaggaatcCATtttcttccatcttc	(12)	<a href="#">(SEQ ID NO 33)</a>
(Phe36Met+Phe99Ala) aagctccacatcGCCgacgagagtggc (13) <a href="#">(SEQ ID NO 34)</a> + primer 12			
(Phe36Met+Phe99Gly) aagctccacatcGCCgacgagagtggc (14) <a href="#">(SEQ ID NO 35)</a> + primer 12			
(Phe36Ala+Phe99Ala) primer 9 + primer 13			
(Phe36Ala+Phe99Gly) primer 9 + primer 14			
Tyr26Ala	caagcatccggtgGCgtgcaccacgcag	(15)	<a href="#">(SEQ ID NO 36)</a>
Asp37Ala	tccgggaggaaGCaaattcttccatc	(16)	<a href="#">(SEQ ID NO 37)</a>

***On page 132, lines 3 - 5, replace the text in the specification with the following;***

Mutant designed for binding to C28/C30 rapalogs:

Glu54Ala      cctcggatcaccGCctgcttgcttag      (17)      (SEQ ID NO 38)

***On page 133, lines 12 - 21, replace the text in the specification with the following;***

Tyr2038His	cctttcccaaagtGcaaacgagatgc	(18) <u>(SEQ ID NO 39)</u>
Tyr2038Leu	cctttcccaaagAGcaaacgagatgc	(19) <u>(SEQ ID NO 40)</u>
Tyr2038Ala	cctttcccaaagGCcaaacgagatgc	(20) <u>(SEQ ID NO 41)</u>
Phe203[8]9His	gttcctttccccAtGgtacaaacgagatg	(21) <u>(SEQ ID NO 42)</u>
Phe203[8]9Leu	gttcctttccccTaagtacaaacgagatg	(22) <u>(SEQ ID NO 43)</u>
Phe203[8]9Ala	gttcctttccccaGCgtacaaacgagatg	(23) <u>(SEQ ID NO 44)</u>
Thr2098Ala	gtcccaggcttggGCgaggtccttgac	(24) <u>(SEQ ID NO 45)</u>
(Lys2095Ser+Asp2096Asn+Thr2098Asn)	gtcccaggcttggTTgaggTTcGAgacattccctgatttc	(25) <u>(SEQ ID NO 46)</u>
Thr2098Asn	gtcccaggcttggTTgaggtccttgac	(26) <u>(SEQ ID NO 47)</u>
Asp2102Ala	catgataatagaggGCccaggcttgggtg	(27) <u>(SEQ ID NO 48)</u>

***On page 134, lines 1 - 6, replace the text in the specification with the following;***

PCR primers (restriction sites upper case; 5'->3'):

gcatcCCATGGcaatcctctggcatgagatgtggcatgaaggcctggaag	(28) <u>(SEQ ID NO 49)</u>
cgtgaGGATCCtactttgagattcgtcggaaacac	(29) <u>(SEQ ID NO 50)</u>

gcatcTCTAGAatcctctggcatgagatgtggcatgaaggcctggaag (30) [\(SEQ ID NO 51\)](#)  
ggtctGGATCCctaataACTAGTctttgagattcgctcggaacacatg (31) [\(SEQ ID NO 52\)](#)

***On page 136, lines 17 - 27, replace the text in the specification with the following;***

3. Addition of FRBs and an epitope tag to pCM generates pCMFR1/2/3.Flag.

A Spel-Flag-BamHI cassette can be prepared by annealing complementary oligonucleotides (oligonucleotides 8 and 9). This cassette has the same features as the Spel-HA-BamHI cassette described above with the exception that the inframe Spel site is followed by sequence that codes for eight amino acids (DYKDDDDY, [SEQ ID NO 53](#)) (Hopp et al., 1988. Biotech. 6: 1205-1210) that is recognized by a monoclonal antibody anti-FLAG.M2 (Kodak Scientific Imaging Systems). The Spel-Flag-BamHI cassette is sub cloned into the Spel/BamHI site in pCGNN-1FRB, pCGNN-2FRB and, pCGNN-3FRB. Subsequently 1/2/3 copies of FRB domain-Flag epitope fusions are sub cloned as a XbaI/BamHI fragment into pCM. The resulting plasmid (pCMFR1/2/3.Flag) has the following features: myristylation domain; an inframe XbaI site; one/two/three copies of FRB; an inframe Spel site; a Flag epitope tag; and stop codons.

***On page 138, lines 1 - 31, replace the text in the specification with the following;***

7. Oligonucleotide sequences

1: CATGTCTAGAGGGAGTAGCAAGAGCAAGCCTAAGGACCCCAGCCAG  
CGCACTAGTTAAGAATTCTGATGATCAGCGGATCCTAGC [\(SEQ ID NO 54\)](#)  
2: GCTAGGATCCGCTGATCATCAGAATTCTTAAGTAGTG  
CGCTGGCTGGGGTCCTTAGGCTTGCTCTTGCTACTCC CTCTAGACATG [\(SEQ ID NO 55\)](#)  
3: CGCCTTGTAGAATTCGCGCGTATGGGGAGTAGCAAGA [\(SEQ ID NO 56\)](#)  
4: CCCAGCCAGCGCTCTAGATAAGAATTCTGA [\(SEQ ID NO 57\)](#)

5: AAGGGTCCCCAACTCAC (SEQ ID NO 58)

6: GCATGACTAGTTATCCGTACGACGTACCAGACT  
ACGCATAAGAAAAGTGAGGATCCTACGG (SEQ ID NO 59)

7: CCGTAGGATCCTCACTTTTCTTATGCGTAGTCTGGT  
ACGTCGTACGGATAACTAGTCATGC (SEQ ID NO 60)

8: CCGTAGGATCCTCACTTTTCTTAATAATCGTCATCG  
TCTTTGTAGTCACTAGTCATGC (SEQ ID NO 61)

9: GCATGACTAGTGACTACAAAGACGATGACGATTA  
TTAAGAAAAGTGAGGATCCTACGG (SEQ ID NO 62)

8. Sequence 1:

CGC CTT GTA GAA ttc GCG CGT ATG ggg agt agc aag agc aag cct aag

D P S Q R S R stop stop (SEQ ID NO 63)

gac ccc agc cag cgc tct aga taa gaa ttc tga tga tca gcG GAT

CCT

GAG AAC T (SEQ ID NO 64)

The modified sequences are in lowercase bold and the initiating ATG is underlined. Sequences in uppercase are from the parental pCG backbone.

Respectfully submitted,



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